

## Cryopreserved mesenchymal stromal cell treatment is safe and feasible for severe dilated ischemic cardiomyopathy

SZE-PIAW CHIN<sup>1</sup>, ALFRED C. POEY<sup>2</sup>, CHEE-YIN WONG<sup>3</sup>, SAU-KONG CHANG<sup>2</sup>, WILLIAM TEH<sup>2</sup>, TEDDRIC JON MOHR<sup>2</sup> & SOON-KENG CHEONG<sup>1</sup>

<sup>1</sup>International Medical University, Kuala Lumpur, Malaysia, <sup>2</sup>Penang Adventist Hospital, Penang, Malaysia, and <sup>3</sup>Cytopeutics, Selangor, Malaysia

### Abstract

**Background aims.** Bone marrow (BM) mesenchymal stromal cells (MSC) represent a novel therapy for severe heart failure with extensive myocardial scarring, especially when performed concurrently with conventional revascularization. However, stem cells are difficult to transport in culture media without risk of contamination, infection and reduced viability. We tested the feasibility and safety of off-site MSC culture and expansion with freeze-controlled cryopreservation and subsequent rapid thawing of cells immediately prior to implantation to treat severe dilated ischemic cardiomyopathy. **Methods.** We recruited three consecutive patients with end-stage ischemic heart failure with evidence of full-thickness myocardial scarring. MSC was isolated from 20 mL BM aspiration, expanded and cryopreserved using 10% dimethyl sulfoxide (DMSO). Cells were transported in a cryoshipper. Patients underwent concurrent coronary artery bypass graft (CABG) with intramyocardial MSC injection. **Results.** The cell viability after thawing exceeded 90% for all samples. The supernatant was free from bacterial and fungal growth. All patients underwent the procedure safely. There were no arrhythmias noted. There was significant improvement in cardiac function and volume, resolution of scarring and increased wall thickness for all patients on cardiac magnetic resonance imaging at 6 months compared with baseline. The magnitude of improvement was more than was expected with CABG alone. Patients remained well at 1 year. **Conclusions.** Rate-controlled freezing with 10% DMSO is a safe, feasible and practical method of cryopreserving MSC for cell storage and transportation without risk of contamination or cell death. Direct MSC injection may be beneficial as an adjunct to cardiac revascularization.

**Key Words:** cryopreservation, heart failure, ischemic cardiomyopathy, mesenchymal stromal cell

### Introduction

Cell therapy is a promising new option for patients with myocardial infarction and severe intractable heart failure (1–3). Mesenchymal stromal cells (MSC) that are found in the bone marrow (BM) have been shown to benefit ischemic cardiomyopathy by several mechanisms, including cardiac cell regeneration, scar tissue resolution, formation of supportive collagen matrix, angiogenesis and paracrine action, that hasten recovery from inflammation (4–9). In one study, intracoronary infusion of MSC for patients who had suffered acute myocardial infarction apparently improved left ventricular function, restored movement of the infarcted regions and reduced scar tissues and left ventricular volumes (7).

Among the limitations of clinical trials is the difficulty in transporting stem cells out of the cell culture facility to operating theatres and hospitals. Commonly in clinical trials, the cells are kept

in culture flasks in constant-controlled incubators until required, when they are then removed from the flasks and resuspended in vials or syringes to be transported to the adjacent operating theatre. While such a workflow is convenient for combined clinical research institutions, it would not be practical for other hospitals without on-site or nearby cell-culture facilities, as not only could the cells become infected or contaminated during transportation but changes in temperature, humidity and oxygen content will also harm the cells. For the same reason, once the stem cells have been removed from the incubator and culture flasks, the procedure or decision to proceed cannot be changed or delayed. Yet delays and postponements in operations are inevitable. Therefore the current method of providing and transporting stem cells is not practical for wider applications.

One practical solution around this problem is to cryopreserve the stem cells upon attaining the numbers required and transport the cells in this state to

the operation theatre and thaw only when required. Cryopreserved stem cells have been in clinical practice for some time now, especially hematopoietic cells for the treatment of leukemia and lymphoma (10–13). However, these cryopreserved cells are normally infused intravenously so the small amounts of cryopreservative used, such as dimethyl sulfoxide (DMSO), are insignificant compared with the intravascular volume. There are a few articles that have reported that cryopreserved BM-derived MSC can retain its expansion and differentiation properties (14–17). However, there is scarce literature on the use of cryopreserved BM-derived MSC for clinical treatment of cardiomyopathy either as transcatheter coronary intervention or the preferred method of direct intramyocardial injection. Therefore providing proof that DMSO-added cryopreserved MSC is safe and feasible for direct intramyocardial injection for patients with heart failure can serve as the first step towards establishing the ‘missing link’ between clinical stem cell trials conducted in university hospitals to translational therapy where the cells produced from one site may be transported safely for use at a remote site.

In this pilot study, we tested the safety and feasibility of using cryopreserved MSC for the treatment of patients with severe intractable heart failure undergoing a concurrent cardiac bypass operation with direct intramyocardial injection.

## Methods

### *Patient selection*

Patients were recruited from a combined cardiology–cardiothoracic clinic (Table I). The inclusion criteria were age between 55 and 75 years old, symptomatic heart failure [New York Heart Association (NYHA) functional classes II–IV] despite optimal medications, previous myocardial infarction (MI) between 3 and 24 months previously, documented left ventricular dysfunction by echocardiography, documented fixed defects or irreversible myocardial scarring on magnetic resonance imaging (MRI) or positron emission tomography (PET) scans, and regarded as high-risk outweighing benefit of revascularization by at least two qualified physicians. The contraindications included any contraindication to BM biopsy, angioplasty or cardiac bypass operation, any acute or chronic intercurrent illnesses such as infections, severe renal failure (serum creatinine greater than 200  $\mu\text{M}$ ), liver failure (transaminase greater than  $2 \times \text{ULN}$ ) and any past or current cancers. All patients signed an extensive informed consent form and gave permission for their data to be published. The study had the approval of the local hospital medical and ethics board.

### *BM aspiration and MSC culture*

We obtained 20 mL BM aspirate (BMA) from each patient using a 14-G Jamshidi needle inserted in the superior posterior iliac crest under local anesthesia. The BMA was collected into pre-heparinized tubes and transferred immediately to the cell culture facility to be processed. Processing time from collection ranged from 4 to 12 h.

BMA was diluted 1:1 with phosphate-buffered saline (PBS) (Gibco, Grand Island, NY, USA) and layered over an equal volume of Ficoll–Paque Premium 1.073 solution (Amersham Biosciences, Uppsala, Sweden). The sample was centrifuged at 400  $g$  for 40 min. Mononuclear cells (MNC) were recovered from the gradient interface and washed with PBS before being resuspended in Dulbecco’s modified Eagle’s medium containing low glucose (DMEM-LG; Gibco) supplemented with 10% autologous serum, 100 U/mL penicillin, 100 mg/mL streptomycin, 250 ng/mL amphotericin B and 2 mM GlutaMAX (Gibco). The cultures were maintained at 37°C in a humidified atmosphere of 5%  $\text{CO}_2$  and 95% air.

After 3 days, non-adherent cells were discarded. Fresh culture medium was replaced every few days until cells reached confluence. The adherent cells were harvested after briefly incubating with TrypLE Select (Gibco) and replated at low density, thus expanding the population of MSC. MSC were confirmed by the presence and absence of several surface adhesions molecules and their ability to differentiate into adipocytes, chondrocytes and osteocytes, as described previously (18,19).

### *MSC cryopreservation and final preparation*

When the required number of cells was reached, MSC were harvested and cryopreserved in 90% autologous plasma and 10% DMSO (Cryoserv, Lake Forest, IL, USA). The cells were frozen gradually using a rate-controlled freezer (Thermo Fisher Scientific, Marietta, OH, USA) to  $-90^\circ\text{C}$ , whereupon they were transferred into vapor phase liquid nitrogen storage. Small volumes of cell culture media that had been used for MSC culture were sent for bacterial and fungal tests.

One day before MSC injection, cryopreserved MSC were transferred to the hospital in a cryoshipper. At the operating theatre, the cells were thawed in a 37°C warm water bath, washed once and then resuspended using sterile 0.9% normal saline solution. The MSC suspension was transferred into a 10 mL luer-lock syringe with a 27-G needle, ready for injection. The duration from cryopreservation to day of surgery was 5–16 days. The viability of the cells in the final suspension was more than 90% in all three cases. The duration from cell resuspension to injection was 1–3 h (Figure 1).

Table I. Characteristics of patients receiving cryopreserved autologous BM-derived MSC by direct intramyocardial injection concurrently with cardiac bypass operation.

	Case 1	Case 2	Case 3
Age (years)	61	63	64
Gender	Male	Male	Male
Number of heart attacks	3	2	2
Most recent heart attack	6 months	5 months	1 month
Diabetes	Yes	Yes	Yes
Stroke	Yes	Yes	No
Hypertension	Yes	Yes	Yes
Chronic renal failure	No	Moderate	No
Number of CAD	3	3	3
Number of CABG	4	4	3
Duration in cryopreservation	5 days	16 days	5 days
Number of MSC injected ( $\times 10^6$ )	28	21	35
Number of injection sites	20	20	20
NYHA baseline	IV	III	IV
NYHA 6 months	I	I	I
LVEF by MRI baseline (%)	26	28	37
LVEF by MRI 6 months (%)	37	49	56
LVEF by echo baseline (%)	21	37	32
LVEF by echo 6 months (%)	56	54	59
LVEDD baseline (mm)	6.04	5.80	5.99
LVEDD 6 months (mm)	5.31	5.05	4.5
LVESD baseline (mm)	5.22	4.86	5.05
LVESD 6 months (mm)	3.74	3.43	3.2

CAD, coronary artery disease; NYHA, New York Heart Association Functional Class (I, asymptomatic; IV, symptomatic at rest); LVEF, left ventricular ejection fraction by echocardiography (echo) or by cardiac MRI (normal 50–75%); LVEDD, left ventricular end diastolic diameter by echocardiography only (normal 35–56 mm); LVESD, left ventricular end systolic diameter by echocardiography only (normal 20–40 mm).

#### *Intramyocardial injection*

Before the operation, patients underwent cardiac perfusion imaging to confirm the presence and extent of scarred or non-viable areas. This was confirmed visually at open heart surgery. Following completion of coronary grafting, the aortic cross clamp was released and blood allowed to fill the ventricle. The MSC suspension was then injected around the scar tissue in a circumference (20). Typically 15–20 sites spaced 1–2 cm apart were injected using a 27-G needle. At each site, 0.5–1.0 mL MSC suspension was injected and light pressure applied after withdrawal of needle.

#### *Follow-up*

Patients remained in hospital for 1–2 weeks. They were monitored in the cardiothoracic intensive care unit by telemetry during the first 48 h for any ventricular arrhythmias. At the end of the hospital stay, patients had an echocardiography to exclude pericardial effusions. They were required to return for follow-up at 6 weeks, 3 months, 6 months and 12 months. Echocardiography to evaluate left ventricular ejection fraction (LVEF), left ventricular end diastolic diameter (LVEDD), left ventricular

end systolic diameter (LVESD), interventricular septal thickness at diastole (IVSd) and regional wall motion index (RWMI) was performed at each visit and compared with baseline values. Cardiac MRI with gadolinium delay enhancement was performed at baseline and 6 months to look for scarring and non-viable areas.

## **Results**

### *Case 1*

A 61-year-old man with three previous MI, stroke with residual hemiparesis and slurred speech, diabetes (HbA1c 7.5) and hypertension presented with severe heart failure (NYHA IV). His LVEF was 20.8% by echocardiography and 26.0% by MRI. MRI further revealed extensive non-viable scarred areas at the anterior, septal and apical segments, with a large antero-apical aneurysm. Thirteen milliliters of BMA were obtained under local anesthetic, which yielded about 20 million MNC. After 4 weeks, we obtained 28 million MSC. The cells were delivered to the hospital 5 days later. The patient underwent quadruple coronary artery bypass grafting (CABG). Athrectomy was also performed to the left anterior descending (LAD) artery. MSC was injected directly into the myocardium

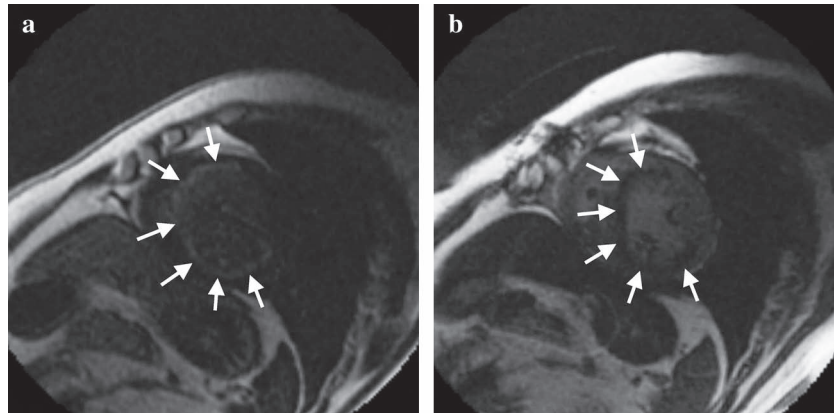


Figure 1. Cardiac magnetic resonance imaging at mid-segment with delayed enhancement of gadolinium for patient 1 at baseline (a) and 6 months (b). (a) The picture shows a white ring along the inner subendocardial border of the heart, which represents scarring (indicated with arrows). (b) Resolution of the scarring as evidenced by the absence of delayed enhancement and an increase in muscle thickness of the myocardium (indicated with arrows).

around the extensive scar tissue. Twenty injections of 0.5 mL each were performed. There were no arrhythmias documented throughout the cardiac monitoring. The patient made an uneventful recovery and at 6 weeks reported improvement in functional class and symptoms (NYHA I-II) and LVEF to 55.0% by echocardiography. Repeat echocardiography findings at 6 months showed LVEF of 53.0% by echocardiography and 37.0% by MRI. MRI also showed no more scarring or aneurysm. In addition there was an increase of muscle thickness in all segments of the left heart, including the previously scarred areas (Figure 1).

#### Case 2

A 63-year-old man with two previous MI, diabetes (HbA1c 8.2%), hypertension and moderate renal failure (serum creatinine 180  $\mu$ M) presented with heart failure (NYHA III) and angina pectoris (CCS II). His LVEF was 37.0% by echocardiography and 28% by MRI. MRI further revealed extensive non-viable scarred areas at the inferior, posterior and apical segments. Twenty milliliters of BMA were obtained under local anesthetic, which yielded about 32 million MNC. After 4 weeks of cell culture, we obtained 21 million MSC. The cells were delivered to the hospital 16 days later. The patient underwent quadruple CABG. MSC was injected directly into the myocardium around the extensive scar tissue. Twenty injections of 0.5 mL each were performed. There were no arrhythmias documented throughout the cardiac monitoring. The patient was discharged after 2 weeks but was re-admitted 1 month later with hemorrhagic gastritis, most probably caused by anti-platelet therapy. Despite the complication, there was improvement in functional class and symptoms (NYHA II) and LVEF to 54.0% by echocardiography. Repeat echocardiog-

raphy findings at 6 months showed further LVEF improvement to 50.0%, 49.0% by MRI.

#### Case 3

A 64-year-old man presented with three previous MI between 1994 and 2008, dyslipidemia, diabetes (HbA1c 7.2%) and hypertension with severe heart failure (NYHA IV). His LVEF was 32.0% by echocardiography and 37.0% by MRI. MRI further revealed extensive non-viable scarred areas at the anterior, septal, lateral, inferior and posterior segments, with a large antero-apical aneurysm. Twenty-seven milliliters of BMA were obtained under local anesthetic, which yielded about 34 million MNC. After 4 weeks, we obtained 35 million MSC. The cells were delivered to the hospital 5 days later. The patient underwent quadruple CABG. MSC was injected directly into the myocardium around the extensive scar tissue at the anterior, septal and apical segments only. Twenty injections of 0.5 mL each were performed. There were no arrhythmias documented throughout the cardiac monitoring. The patient made an uneventful recovery and at 6 weeks reported improvement in functional class and symptoms (NYHA I-II) and LVEF to 52.4% by echocardiography. Repeat echocardiography findings at 6 months showed further LVEF improvement to 53.0% by echocardiography and 58.0% by MRI. MRI also showed reduced scarring at the anterior and apical segments but no reduction of scarring in the areas not previously injected.

#### Discussion

The ability to store stem cells in a dormant, stable and sterile state for a period of time prior to transplantation is important for practical clinical applications,

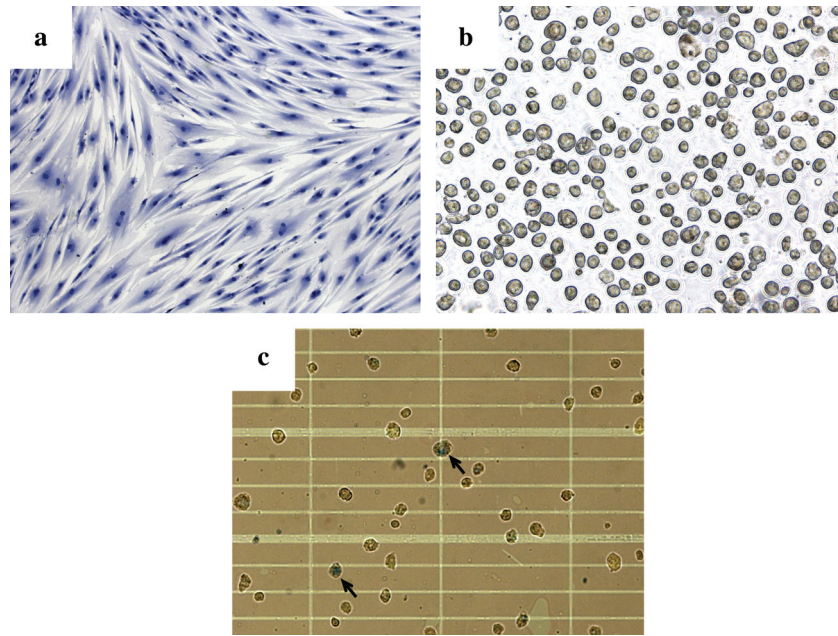


Figure 2. (a) Morphology of MSC. (b) Suspension of MSC in 0.9% normal saline solution at a concentration of  $3 \times 10^6$  cell/mL. (c) Staining of MSC with trypan blue shows a >90% cell viability after cryopreservation, rapid thawing and resuspension (dead cells were stained blue, as indicated with arrows).

one that will allow stem cells to be transported over large distances safely. Keeping the cells cryopreserved all the way to the operating theatre or clinic and thawing the cells on site only when required circumvents the uncertainty of the timing of stem cell injection. Furthermore, this process also ensure the quality and sterility of the cells. Quality checking (QC) is a vital step in the production of stem cells for clinical applications (20). At a finite point when the cells have attained the necessary number, the cells are removed from culture and tested for purity, viability and sterility. As some of the QC, such as bacterial testing, typically takes 72–108 h to confirm, the cells must be preserved in a dormant state. During this time, it is important to ensure that the cells are not vulnerable to possible contamination of infection or undergo any further expansion or differentiation. Hence cryopreservation is critical to the reliability of QC.

Currently, *ex vivo*-expanded MSC have been used in many clinical trials, for example to treat graft-versus-host disease (GvHD) (21), stroke (22), spinal cord injury (23), osteogenesis imperfecta (24,25) and osteoarthritis (26). None of these trials has shown any side-effect of MSC after transplantation. All of these trials show that MSC are safe and feasible for treatment even after *ex vivo* expansion. However, it is recommended that DMSO-containing cryopreserved MSC are washed before transplantation.

In the cryopreservation process, the most common cryopreservative used routinely for storage of cells is DMSO. DMSO is known to have toxic side-

effects and also potentially cause hypersensitivity reactions (27–29). There are also reports of acute encephalopathy, yet guidelines for their use in stem cell transplantation do not exist. In 2004, the European Group for Blood and Transplantation (EBMT) carried out a questionnaire survey on DMSO toxicity in autologous stem cell transplantation (30). Out of 34 000 transplants, at least 470 cases of toxicity were reported and almost half of the cases were cardiovascular in nature. Moreover, it was reported that the incidence of toxicity was significantly lower when cells were washed before transplantation. Thus, it is recommended to wash DMSO-containing cryopreserved cells before transplantation. For our method of direct intramyocardial injection, the cells need to be washed quickly and resuspended in sterile normal saline solution. Typically, for a 2-mL cryovial containing up to 10 million MSC, we add 10% DMSO (0.2 mL) and 90% autologous plasma (1.8 mL) for cryopreservation. At the end of this final cell preparation, after a single wash and resuspension, the DMSO content is less than 1% in a 10-mL syringe containing up to 30 million MSC (Figure 2).

As all three case studies illustrate, intramyocardial injection of stem cells is feasible and safe. They also illustrate significant improvement in heart contractibility and function, with resolution of symptoms and myocardial scarring. The magnitude of improvement in heart contractibility by an average of 20% points, which exceeds that of either CABG or angioplasty revascularization procedures alone,

was reported consistently as 10% after 6 months. We used two methods of LVEF measurement, and MRI is regarded as superior and is currently the gold standard. With MRI, an LVEF improvement of 5% is regarded as significant recovery, whereas our patients demonstrated 10–20% point improvement. Furthermore, there was normalization of the left ventricular end diastolic and systolic diameters, which indicates reduction in dilatation and scarring. These results are similar to other published literature using either BM stem cells or more specifically MSC (1–9). All the more remarkable is that all three patients were deemed previously to have end-stage heart failure and be unlikely to benefit from revascularization strategy alone because of non-viable scars, and all remained well at up to 12 months follow-up.

The three cases in this pilot study have demonstrated the safety and feasibility of direct intramyocardial injection of cryopreserved MSC. Cryopreserving MSC in a rate-controlled manner does not lead to significant cell death and is an important practical consideration for storage, transportation and quality assurance. The cryopreserved MSC may be efficacious for the treatment of severe intractable heart failure.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## References

1. Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, et al. Adult bone marrow derived cells for cardiac repair: a systematic review and metaanalysis. *Arch Int Med* 2007;167:989–97.
2. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Eng J Med* 2006;355:1210–21.
3. Assmus B, Schachinger V, Teupe C, Britten M, Lahmann R, Dobert N, et al. Transplantation of progenitor cells and revascularization enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 2002;106:3009–17.
4. Pittenger MF, Bradley JM. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* 2004;95:9–20.
5. Minguell JJ, Ericas A. Mesenchymal stem cells and the treatment of cardiac disease. *Exp Biol Med* 2006;231:39–49.
6. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 2002;105:93–8.
7. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intra-coronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 2004;94:92–5.
8. Katritsis DG, Sotiropoulou PA, Karvouni E, Karabinos I, Karothesis S, Perez SA, et al. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Interv* 2005;65:321–9.
9. Mohyeddin-Bonab M, Mohamad-Hasani MR, Alimogaddam K, Sanatkar M, Gasemi M, Mirhhani H, et al. Autologous *in vitro* expanded mesenchymal stem cell therapy for human old myocardial infarction. *Arch Iranian Med* 2007;10:467–73.
10. Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AR, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci USA* 1995;92:10119–22.
11. Meyer TPH, Hofmann B, Zaisserer J, Jacobs V, Fuchs B, Rapp S, et al. Analysis and cryopreservation of hematopoietic stem and progenitor cells from umbilical cord blood. *Cytotherapy* 2006;8:265–76.
12. Galmes A, Gutierrez A, Sampol A, Canaro M, Morey M, Iglesias J, et al. Long-term hematologic reconstitution and clinical evaluation of autologous peripheral blood stem cell transplantation after cryopreservation of cells with 5% and 10% dimethylsulfoxide at –80°C in a mechanical freezer. *Haematologica* 2007;92:986–9.
13. Nagamura-Inoue T, Shioya M, Sugo M, Cui Y, Takahashi A, Tomita S, et al. Wash-out of DMSO does not improve the speed of engraftment of cord blood transplantation: follow-up of 46 adult patients with units shipped from a single cord blood bank. *Transfusion* 2003;43:1285–95.
14. Haack-Sorensen M, Bindsvlev L, Mortensen S, Friis T, Kastrup J. The influence of freezing and storage on the characteristics and functions of human mesenchymal stromal cells isolated for clinical use. *Cytotherapy* 2007;9:328–37.
15. Casado-Diaz A, Santiago-Mora R, Jimenez R, Caballero-Villarraso J, Herrera C, Torres A, et al. Cryopreserved human bone marrow mononuclear cells as a source of mesenchymal stromal cell: application in osteoporosis research. *Cytotherapy* 2008;10:460–8.
16. Reger RL, Wolfe MR. Freezing harvested hMSCs and recovery of hMSCs from frozen vials for subsequent expansion, analysis, and experimentation. *Methods Mol Biol* 2008;449:109–16.
17. Samuelsson H, Ringden O, Lonnie H, Le Blanc K. Optimizing *in vitro* conditions for immunomodulation and expansion of mesenchymal stromal cells. *Cytotherapy* 2009;11:129–36.
18. Wong CY, Cheong SK, Mok PL, Leong CF. Differentiation of human mesenchymal stem cells into mesangial cells in post-glomerular injury murine model. *Pathology* 2008;40:52–7.
19. Mok PL, Leong CF, Cheong SK. Isolation and identification of putative mesenchymal stem cells from bone marrow. *Malays J Pathol* 2003;25:121–7.
20. Law PK, Chin SP, Hung HD, Nguyen TN, Feng QZ. Delivery of biologics for angiogenesis and myogenesis. In: Nguyen TN, Colombo A, Hu D, Grines CL, Saito S. Practical Handbook of Advanced Interventional Cardiology, 3rd edn. Blackwell; 2008. p. 583–95.
21. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 2008;371:1579–86.
22. Bang YO, Lee SJ, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol* 2005;57:874–82.
23. Moviglia GA, Fernandez Viña R, Brizuela JA, Saslavsky J, Vrsalovic F, Varela G, et al. Combined protocol of cell therapy for chronic spinal cord injury. Report on the electri-

- cal and functional recovery of two patients. *Cytotherapy* 2006;8:202–9.
24. Le Blanc K, Gottherstrom C, Ringden O, Hassan M, McMahon R, Horwitz E, *et al.* Fetal mesenchymal stem-cell engraftment in bone after *in utero* transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 2005;79:1607–14.
  25. Horwitz EM, Gordon PL, Koo WKK, Marx JC, Neel MD, McNall RY, *et al.* Isolated allogeneic bone marrow-derived mesenchymal stem cells engraft and stimulate growth in children with osteogenesis imperfecta: implication for cell therapy of bone. *Proc Natl Acad Sci USA* 2002;99: 8932–7.
  26. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal stem cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002;1:199–206.
  27. Petropoulou AD, Bellochine R, Norol F, Marie JP, Rio B. Coronary artery spasm after infusion cryopreserved cord blood cells. *Bone Marrow Transplant* 2007;40:397–8.
  28. Hubel A. Cryopreservation of HPCs for clinical use. *Transfusion* 2001;41:579–80.
  29. Syme R, Bewick M, Stewart D, Porter K, Chadderton T, Glück S. The role of depletion of dimethyl sulfoxide before autografting: on hematologic recovery, side effects, and toxicity. *Biol Blood Marrow Transplant* 2004;10:135–41.
  30. Windrum P, Morris TCM, Drake MB, Niederwieser D, Ruutu T. Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres. *Bone Marrow Transplant* 2005;36:601–3.